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Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

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1. Introduction

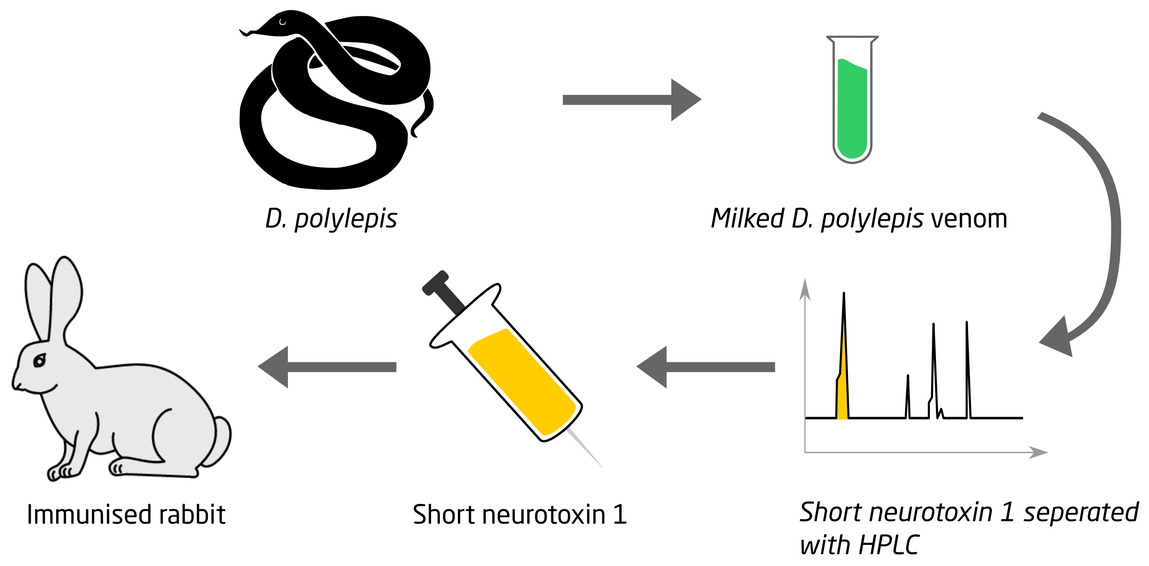
The black mamba (*Dendroaspis Polylepis*) is among the most dangerous snakes in the world, with a venom dominated by three-finger toxins and dendrotoxins[1]. Among the three-finger toxins, the α-neurotoxins (α-NT) are the most important[2], and these are conserved between snake species.

Cross-reactivity between three-finger toxins is known to occur, and understanding this phenomenon in depth may help guide future design of antivenoms to obtain optimal specificity against medically important toxins from different snake species.

Using a bioinformatic approach, we investigated the cross-reactivity between three-finger toxins for a rabbit antiserum raised against short neurotoxin 1 from *D. polylepis* (SN1-DP).

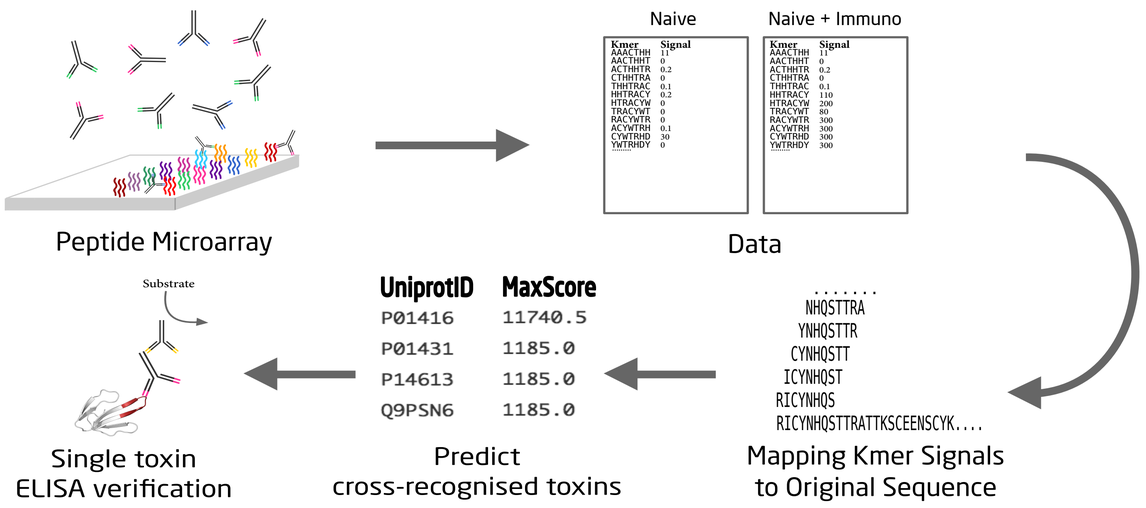
2. Immunisation

The venom of *D. polylepis* was obtained, SN1-DP was isolated and used for immunisation of a rabbit.



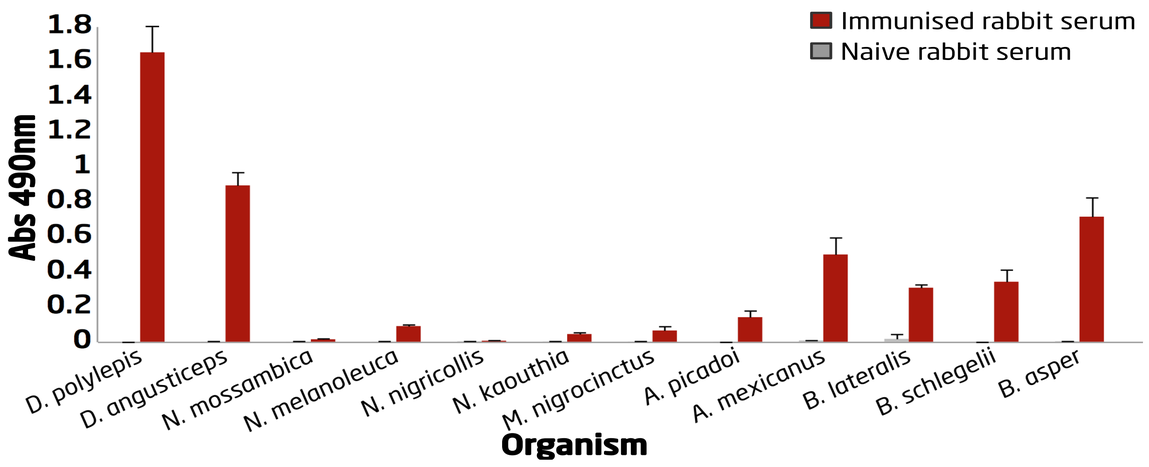
3. Project Flow

A peptide microarray covering all possible epitopes of all known three-finger toxins was synthesised, and binding of antibodies from the rabbit serum to individual toxin epitopes was detected and mapped back to toxin structures.



4. ELISA

An ELISA experiment containing various snake venoms was performed using the rabbit antisera immunised with SN1-DP. This shows reactivity with *D. polylepis* whole venom, strong cross-reactivity with the related *D. angusticeps* venom, and some cross-reactivity with very unrelated venoms.



5. Mapped Signals

A sequence markup plot was constructed for all toxins within the same sub-subfamily of positive control SN1-DP (UniprotID: P01416).

Comparing the high antibody binding regions of SN1-DP and the second highest scoring toxin it shows the that *Y28R* possibly has a high impact, which also confirms that Tyrosine (Y) is more common in epitopes than Arginine (R)[3]. But it does look like it maintains its signal downstream even with the mutations *TII35:37YRT*. Due to the normalisation of each sequence, it should be noted that all marked positions in P01416 have a larger signal than any signals in the other toxins.

$$AAsignal = \sum_{k=\{8,12,15\}} AA \in k_{mer} \qquad MaxScore = max(AAsignals)$$

UniprotID	Organism	MaxScore	Sequence
P01416	*D. polylepis*	11740.5	RICYNHQSTTRATTKSC--EENSCYKKYWRDHRGTIIERGCGCPKVKPGVGIHCCQSDKCNY
P01431	*N. mossambica*	1185.0	LECHNQQSSEPPTTTRCSGGETNCYKKRWRDHRGYRTERGCGCPTVKKGIELNCCTTDRCNN
P14613	*N. kaouthia*	1185.0	LECHNQQSIQTPTTTGCSGGETNCYKKRWRDHRGYRTERGCGCPSVKNGIEINCCTTDRCNN
Q9PSN6	*N. sputatrix*	1185.0	LECHDQQSSQTPTTTGCSGGETNCYKKRWRDHRGYRTERGCGCPSVKNGIEINCCTTDRCNN
P60770	*N. atra*	1185.0	LECHNQQSSQTPTTTGCSGGETNCYKKRWRDHRGYRTERGCGCPSVKNGIEINCCTTDRCNN
P60771	*N. kaouthia*	1185.0	LECHNQQSSQTPTTTGCSGGETNCYKKRWRDHRGYRTERGCGCPSVKNGIEINCCTTDRCNN
Q9PTT0	*N. naja*	1185.0	LECHNQQSSQTPTTTGCSGGETNCYKKRWRDHRGYRTERGCGCPSVKNGIEINCCTTDRCNN
Q9YGJ6	*N. sputatrix*	1110.0	LECHNQQSSETPTTTGCSGGETNCYKKSWRDHRGYRIERGCGCPSVKKGIEINCCTTDRCNN
Q9YGJ5	*N. sputatrix*	1110.0	LECHNQQSSQAPTTTGCSGGETNCYKKSWRDHRGYRIERGCGCPSVKKGIEINCCTTDRCNN
			1--------10--------20--------30--------40--------50--------60-

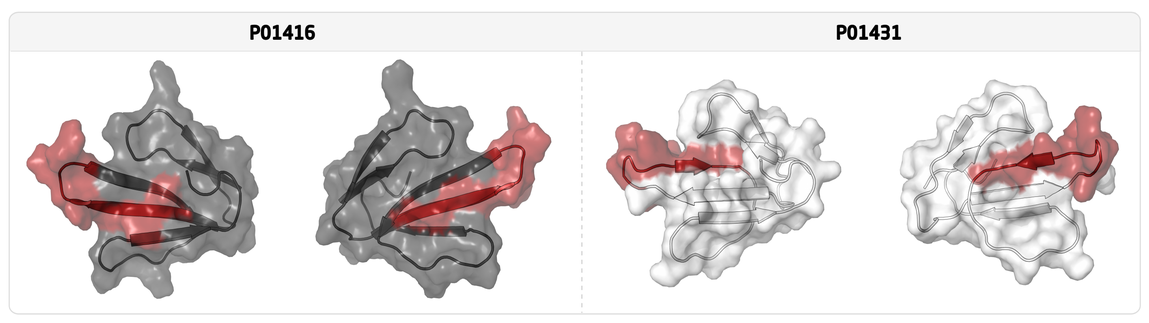
6. Structural Comparison

Due to the observed elimination of the start region on the sequence markup plot, the top toxin (P01431) was modelled due to the lack of a PDB structure using SWISS-MODEL[4].

The structure for SN1-DP (PDB: 1NTX) and the modelled P01431 structure were compared in PyMOL[5], and their amino acid sequences showing a high degree of antibody binding were marked in red.

Red regions:

P01416: RICYNHQSTTRATTKSC--EENSCYKKYWRDHRGTIIERGCGCPKVKPGVGIHCCQSDKCNY
P01431: LECHNQQSSEPPTTTRCSGGETNCYKKRWRDHRGYRTERGCGCPTVKKGIELNCCTTDRCNN



Conclusion & Future

A high chance of antivenom cross-reactivity between short neurotoxins from different snake species exists due to conservation of important epitopes in these toxins. Future ELISA experiments containing SN1-DP and some of the toxins with high signal will be conducted to further support the results observed in this bioinformatic analysis.

References

1: Laustsen et al. Journal of proteomics 119 (2015): 126-142.
2: Laustsen et al. Toxicon 104 (2015): 43-45.
3: Kringelum et al. Molecular immunology 53.1 (2013): 24-34.
4: Biasini et al. Nucleic acids research (2014): gku340.
5: PyMOL Version 1.7.4 Schrödinger, LLC
6: Black Mamba image, Herman Pijpers - www.flickr.com (Adapted)

Acknowledgements

We would like to thank Novo Nordisk Fonden (Grant number: NNF13OC0005613), Denmark, Det Frie Forskningsråd, Lundbeckfonden, Brødrene Hartmanns Fond, Drug Research Academy, Dansk Tennis Fond Oticon Fonden, Knud Højgaards Fond, Rudolph Als Fondet, Henry Shaws Legat, Læge Johannes Nicolai Krigsgaard of Hustru Else Krogsgaards Mindelegat for Medicinsk Forskning og Medicinske Studenter ved Københavns Universitet, Lundbeckfonden, Torben of Alice Frimodts Fond, Frants Allings Legat and Christian og Ottilia Brorsons Rejselegat for Yngre Videnskabsmænd og -kvinder.